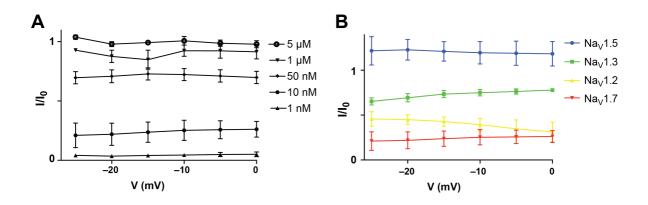
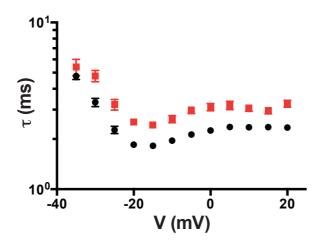
Supporting Material

Seven novel modulators of the analgesic target Na_V1.7 uncovered using a high-throughput venoms-based discovery approach

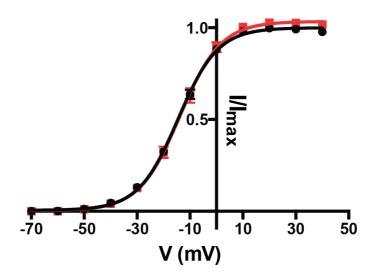
Supplementary Figure 1



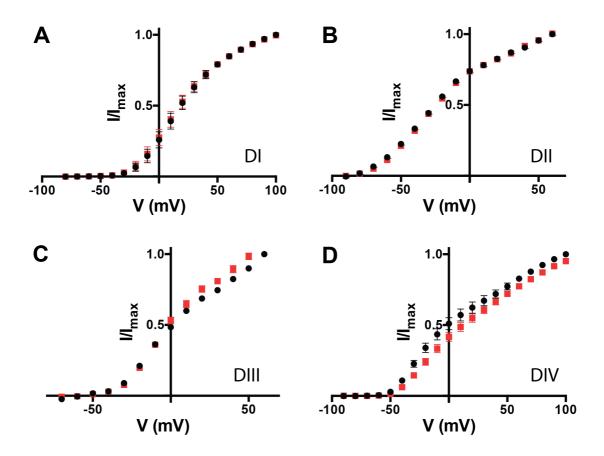
Supplementary Figure 1. Voltage-dependence of Na_V channel inhibition by rHd1a. (A) Inhibition of Na_V1.7-mediated currents by rHd1a is not voltage dependent from -25 mV to 0 mV (n = 3-4; error bars denote s.e.m.). Currents were evoked from a holding potential of -90 mV, stepping from -70 to 0 mV in 5-mV increments. (B) Selectivity of rHd1a is not voltage-dependent from -25 mV to 0 mV. I/I₀ in the presence of 1 μ M rHd1a for hNa_V1.2, hNa_V1.3, hNa_V1.5 and hNa_V1.7 (n = 3-4; error bars denote s.e.m). Currents were evoked from a holding potential of -90 mV, stepping from -70 to 0 mV in 5-mV increments.



Supplementary Figure 2. Effect of rHd1a on hNa_V1.7 inactivation time constants. Time constant (τ) for inactivation of hNa_V1.7 in the absence (black) or presence (red) of 1 μ M rHd1a (n = 5–7; error bars denote s.e.m). The rate of inactivation is slowed in the presence of rHd1a. Currents were evoked from a holding potential of –90 mV, stepping from –70 to +20 mV in 5-mV increments.

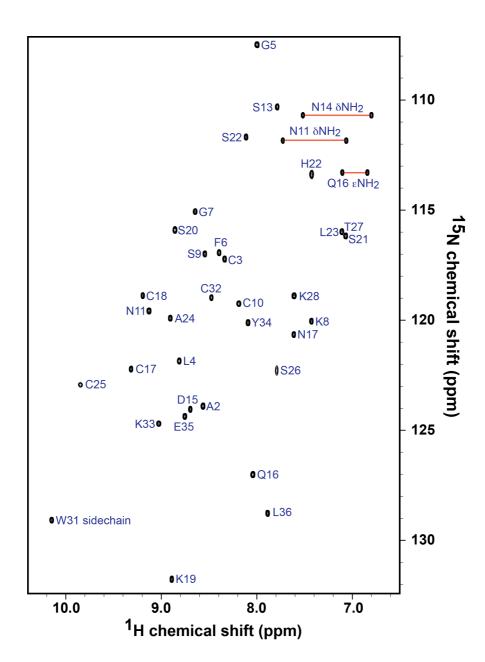


Supplementary Figure 3. Effect of rHd1a on human ERG channels (hERG, Kv11.1). The current–voltage (I–V) relationship for hERG was measured by applying 2-s pulses to test potentials ranging from -70 to +40 mV in 10-mV increments and then by a repolarizing step to -70 mV for 1 s. Tail currents are plotted as a function of voltage and fitted to a Boltzmann equation (n = 6; error bars denote s.e.m). There was no significant difference between control currents (black) and those following addition of 1 μ M rHd1a (red).



Supplementary Figure 4. Effect of rHd1a on hNa_V1.9/rKv2.1 chimeras. Tail current-voltage relationships for a panel of hNa_V1.9/rKv2.1 chimeras obtained before (black) or after (red) addition of 1 μM rHd1a (*n* = 5–8; error bars denote s.e.m.). These chimeras were constructed by transplanting the S3b-S4 paddle from each domain of hNa_V1.9 (DI, DII, DIII and DIV, as indicated) into rKv2.1; construction and characterisation of these chimeras was reported previously¹. The holding voltage was –90 mV (or –120 mV for DII) and the tail voltage was –70 mV for the DI (panel A), –90 mV for DII (panel B), –60 mV for DIII (panel C), and –80 mV for DIV (panel D) chimeras. rHd1a had no effect on any of the chimeric channels.

^{1.} Bosmans F, Puopolo M, Martin-Eauclaire MF, Bean BP, Swartz KJ (2011). Functional properties and toxin pharmacology of a dorsal root ganglion sodium channel viewed through its voltage sensors. *J. Gen. Physiol.* 138: 59–72.



Supplementary Figure 5. Fully assigned 2D ¹H-¹⁵N-HSQC spectrum of Hd1a. Horizontal lines connect peaks corresponding to the two sidechain NH groups of N11, N14, and Q16. The peak from the indole sidechain of W31 is also labelled. All other peaks are from backbone amide groups. The ¹H-¹⁵N-HSQC spectrum is missing resonances from the backbone amide groups of K30 and W31, but they were present in 3D ¹⁵N- and ¹³C-edited HSQC-NOESY spectra.

Supplementary Table 1: List of species from which venom was obtained for this study.

Family	Genus	Species	Gender	Continent	hNa _v 1.7 activity ²
Araneidae	Argiope	sp. (Papua New Guinea ¹)	female	Asia	NA
Araneidae	Cyrtophora	moluccensis	female	Asia	NA
Araneidae	Eriophora	transmarina	female	Australia	NA
Atypidae	Atypus	piceus	female	Europe	Inhibitor
Austrochilidae	Hickmania	troglodytes	female	Australia	NA
Ctenidae	Ancylometes	sp. (Guatemala)	female	America	Inhibitor
Ctenidae	Cupiennius	salei	female	America	NA
Ctenidae	Phoneutria	nigriventer	female	America	Agonist
Desidae	Badumna	insignis	female	Australia	Inhibitor
Dipluridae	Linothele	fallax	female	America	NA
Hexathelidae	Atrax	robustus	female	Australia	NA
Hexathelidae	Hadronyche	infensa	female	Australia	Agonist
Hexathelidae	Macrothele	gigas	female	Asia	NA
Idiopidae	Euoplos	sp. (NSW, Australia)	female	Australia	Inhibitor
Lycosidae	Allocosa	obscuroides	female	Asia	NA
Lycosidae	Unidentified	sp. (QLD, Australia)	female	Australia	NA
Nemesiidae	Acanthogonatus	francki	female	America	NA
Nemesiidae	Aname	sp. (QLD, Australia)	female	Australia	NA
Nemesiidae	Aname	sp. (WA, Australia)	female	Australia	NA
Nephilidae	Nephila	pilipes	female	Asia	NA
Nephilidae	Nephila	plumipes	female	Australia	NA
Pisauridae	Dolomedes	sp. (QLD, Australia)	female	Australia	NA
Pisauridae	Megadolomedes	australianus	female	Australia	Inhibitor
Sparassidae	Heteropoda	jugulans	male	Australia	Inhibitor
Sparassidae	Neosparassus	diana	female	Australia	Inhibitor
Sparassidae	Unidentified	sp. 1 (Mission, Papua New Guinea)	male	Asia	Inhibitor
Sparassidae	Unidentified	sp. 2 (Fraser Island, QLD, Australia)	unknown	Australia	Inhibitor
Sparassidae	Unidentified	sp. 3 (Port Moresby, Papua New Guinea)	female	Asia	Inhibitor
Sparassidae	Unidentified	sp. 4 (Regency Downs, QLD, Australia)	female	Australia	Inhibitor
Sparassidae	Unidentified	sp. 5 (Gobe, Papua New Guinea)	female	Asia	Inhibitor
Sparassidae	Unidentified	sp. 6 (Gobe, Papua New Guinea)	female	Asia	Inhibitor
Theraphosidae	Acanthoscurria	antillensis	female	America	NA
Theraphosidae	Acanthoscurria	geniculata	male	America	NA
Theraphosidae	Agnostopelma	tota	female	America	NA
Theraphosidae	<u> </u>	seemanni	male	America	Agonist
Theraphosidae	Aphonopelma	sp. (Vera Cruz, Mexico)	female	America	Agonist
Theraphosidae	Aphonopelma	sp. (Malinche, Mexico)	female	America	NA
Theraphosidae	Avicularia	bicegoi	female	America	NA
Theraphosidae	Avicularia	diversipes	female	America	NA
Theraphosidae	Avicularia	minatrix	female	America	NA
Theraphosidae	Avicularia	purpurea	female	America	NA
Theraphosidae	Avicularia	sp. (amazonica)	female	America	NA
Theraphosidae	Avicularia	sp. (Guyana)	female	America	NA
Theraphosidae	Avicularia	urticans	female	America	NA
Theraphosidae	Avicularia	versicolor	female	America	Inhibitor
Theraphosidae	Bonnetina	tanzeri	female	America	Inhibitor
Theraphosidae	Brachypelma	albopilosum	female	America	NA
Theraphosidae	Brachypelma	annitha	female	America	NA NA
Theraphosidae	Brachypelma	boehmei	female	America	Agonist
Theraphosidae	Brachypelma	klaasi	female	America	NA
		sabulosum			Agonist
Theraphosidae	I Brachuneima				
Theraphosidae Theraphosidae	Brachypelma Brachypelma	schroederi	female female	America America	Agonist

Family	Genus	Species	Gender	Continent	hNa _V 1.7 activity ²
Theraphosidae	Brachypelma	vagans	female	America	Agonist
Theraphosidae	Ceratogyrus	brachycephalus	female	Africa	Inhibitor
Theraphosidae	Ceratogyrus	darlingi	female	Africa	NA
Theraphosidae	Ceratogyrus	marshalli	female	Africa	Inhibitor
Theraphosidae	Ceratogyrus	sanderi	female	Africa	Inhibitor
Theraphosidae	Chaetopelma	olivaceum	female	Africa	Inhibitor
Theraphosidae	Chilobrachys	dyscolus	female	Asia	Agonist
Theraphosidae	Chilobrachys	fimbriatus	female	Asia	NA
Theraphosidae	Chilobrachys	guangxiensis	female	Asia	Agonist
Theraphosidae	Chilobrachys	huahini	female	Asia	NA
Theraphosidae	Chilobrachys	sp. (Malaysia)	female	Asia	NA
Theraphosidae	Chromatopelma	cyaneopubescens	female	America	Inhibitor
Theraphosidae	Coremiocnemis	tropix	female	Australia	Inhibitor
Theraphosidae	Crassicrus	Iamanai	female	America	NA
Theraphosidae	Cyclosternum	fasciatum	female	America	Inhibitor
Theraphosidae	Cyclosternum	sp. (Boyaca, Colombia)	female	America	NA
Theraphosidae	Cyclosternum	sp. (Meta, Colombia)	female	America	NA
Theraphosidae	Cyriapagopus	sp. ("Sulawesi black")	female	Asia	NA
Theraphosidae	Cyriopagopus	schioedtei	female	Asia	Inhibitor
Theraphosidae	Cyrtopholis	ramsi	female	America	NA
Theraphosidae	Encyocratella	olivacea	male	Africa	Agonist
Theraphosidae	Ephebopus	cyanognathus	female	America	NA
Theraphosidae	Ephebopus	murinus	female	America	NA
Theraphosidae	Ephebopus	rufescens	female	America	NA
Theraphosidae	Ephebopus	uatuman	female	America	NA
Theraphosidae	Euathlus	pulcherrimaklaasi	female	America	Inhibitor
Theraphosidae	Euathlus	sp. ("green femur", Chile)	female	America	Inhibitor
Theraphosidae	Euathlus	sp. ("red", Chile)	female	America	Inhibitor
Theraphosidae	Euathlus	truculentus	female	America	NA
Theraphosidae	Eucratoscelus	constrictus	female	Africa	Inhibitor
Theraphosidae	Eupalaestrus	campestratus	female	America	Inhibitor
Theraphosidae	Eupalaestrus	weijenberghi	female	America	NA
Theraphosidae	Grammostola	actaeon	female	America	NA
Theraphosidae	Grammostola	porteri	female	America	Inhibitor
Theraphosidae	Grammostola	pulchra	female	America	Inhibitor
Theraphosidae	Grammostola	pulchripes	female	America	Inhibitor
Theraphosidae	Grammostola	rosea	female	America	Inhibitor
Theraphosidae	Grammostola	sp. ("Chile North")	female	America	Inhibitor
Theraphosidae	Hapalopus	sp. ("Colombia, large")	female	America	NA
Theraphosidae	Haploclastus	nilgirinus	female	America	Agonist
Theraphosidae	Haplopelma	albostriatum	female	Asia	Inhibitor
Theraphosidae	Наріореіта Наріореіта	doriae	male	Asia	Inhibitor
Theraphosidae	Haplopelma	lividum	female	Asia	Inhibitor
Theraphosidae	Наріореіта Наріореіта	minax	female	Asia	Inhibitor
Theraphosidae		schmidti	female	Asia	Inhibitor
Theraphosidae	Haplopelma Haplopelma		female	Asia	NA
Theraphosidae	Haplopelma Harpactira	sp. (Borneo)	male	Africa	
Theraphosidae	Harpactira	cf. gigas guttata	female	Africa	Agonist NA
•	Heteroscodra	1 -			
Theraphosidae		maculata	female	Africa	Agonist
Theraphosidae	Holothele	sp. ("Meta", Colombia)	female	America	NA NA
Theraphosidae	Holothele	sp. ("Norte de Santander", Colombia)	female	America	NA Aganist
Theraphosidae	Holothele	sp. (Choroni, Venezuela)	female	America	Agonist
Theraphosidae	Holothele	sp. (Isla Margarita, Venezuela)	female	America	NA
Theraphosidae	Holothele	sp. (Peru)	female	America	Inhibitor
Theraphosidae	Holothele	sp. (Romerito, Venezuela)	female	America	NA Jalailaitaa
Theraphosidae	Hysterocrates	ederi	female	Africa	Inhibitor

Family	Genus	Species	Gender	Continent	hNa _V 1.7 activity ²
Theraphosidae	Hysterocrates	hercules	female	Africa	Inhibitor
Theraphosidae	Hysterocrates	sp. (Cameroon)	female	Africa	Inhibitor
Theraphosidae	Hysterocrates	sp. (Nigeria)	female	Africa	Inhibitor
Theraphosidae	Hysterocrates	sp. (West Cameroon)	female	Africa	Inhibitor
Theraphosidae	Ischnocolus	valentinus	female	Europe	NA
Theraphosidae	Lampropelma	nigerrimum	female	Asia	NA
Theraphosidae	Lampropelma	violaceopes	female	Asia	Inhibitor
Theraphosidae	Lasiodora	difficilis	female	America	NA
Theraphosidae	Lasiodora	klugi	female	America	NA
Theraphosidae	Lasiodora	parahybana	female	America	NA
Theraphosidae	Megaphobema	robustum	female	America	NA
Theraphosidae	Metriopelma	familiare	female	America	Inhibitor
Theraphosidae	Metriopelma	sp. (Venezuela)	female	America	Inhibitor
Theraphosidae	Monocentropus	balfouri	female	Africa	Inhibitor
Theraphosidae	Monocentropus	lambertoni	female	Africa	NA
Theraphosidae	Nhandu	coloratovillosus	female	America	NA
Theraphosidae	Nhandu	tripepii	female	America	Inhibitor
Theraphosidae	Ornithoctonus	aureotibialis	female	Asia	Inhibitor
Theraphosidae	Ornithoctonus	sp. (Koh Samui, Thailand)	female	Asia	Inhibitor
Theraphosidae	Orphnaecus	pellitus	female	Asia	Inhibitor
Theraphosidae	Orphnaecus	sp. ("treedweller", Philippines)	female	Asia	NA
Theraphosidae	Orphnaecus	sp. (Cebu, Philippines)	female	Asia	Inhibitor
Theraphosidae	Orphnaecus	sp. (Maanghit-Cave, Philippines)	female	Asia	Inhibitor
Theraphosidae	Orphnaecus	sp. (Mt. Combuon, Phillippines)	female	Asia	NA
Theraphosidae	Orphnaecus	sp. (Sibaliw, Panay, Philippines)	female	Asia	Inhibitor
Theraphosidae	Orphnaecus	sp. (Sipalay, Phillippines)	female	Asia	Inhibitor
Theraphosidae	Pamphobeteus	antinous	female	America	Inhibitor
Theraphosidae	Pamphobeteus	fortis	male	America	NA
Theraphosidae	Pamphobeteus	nigricolor	male	America	Inhibitor
Theraphosidae	Pamphobeteus	sp.1 (Colombia)	female	America	NA
Theraphosidae	Pamphobeteus	sp.2 (Colombia)	female	America	NA
Theraphosidae	Paraphysa	parvula	female	America	Inhibitor
Theraphosidae	Phlogiellus	inermis	female	Asia	Inhibitor
Theraphosidae	Phormictopus	auratus	female	America	Inhibitor
Theraphosidae	Phormictopus	cancerides	female	America	Inhibitor
Theraphosidae	Phormictopus	cautus	female	America	NA
Theraphosidae	Phormictopus	cochleasvorax	male	America	Inhibitor
Theraphosidae	Phormingochilus	everetti	female	Asia	Inhibitor
Theraphosidae	Plesiopelma	longisternale	female	America	Inhibitor
Theraphosidae	Plesiopelma	sp. (Bolivia)	female	America	NA
Theraphosidae	Poecilotheria	fasciata	female	Asia	Inhibitor
Theraphosidae	Poecilotheria	formosa	female	Asia	Agonist
Theraphosidae	Poecilotheria	hanumavilasumica	female	Asia	NA
Theraphosidae	Poecilotheria	metallica	female	Asia	Inhibitor
Theraphosidae	Poecilotheria	miranda	female	Asia	Agonist
Theraphosidae	Poecilotheria	ornata	female	Asia	Agonist
Theraphosidae	Poecilotheria	regalis	female	Asia	NA
Theraphosidae	Poecilotheria	rufilata	female	Asia	Agonist
Theraphosidae	Poecilotheria	smithi	female	Asia	Agonist
Theraphosidae	Poecilotheria	striata	female	Asia	NA
Theraphosidae	Poecilotheria	subfusca	female	Asia	Agonist
Theraphosidae	Poecilotheria	tigrinawesseli	male	Asia	Agonist
Theraphosidae	Poecilotheria	vittata	female	Asia	NA
Theraphosidae	Psalmopoeus	cambridgei	female	America	NA
Theraphosidae	Psalmopoeus	irminia	female	America	Inhibitor
Theraphosidae	Psalmopoeus	langenbucheri	female	America	NA

Family	Genus	Species	Gender	Continent	hNa _V 1.7 activity ²
Theraphosidae	Psalmopoeus	pulcher	female	America	Inhibitor
Theraphosidae	Psalmopoeus	reduncus	female	America	NA
Theraphosidae	Pseudhapalopus	spinulopalpus	male	America	NA
Theraphosidae	Pterinochilus	chordatus	female	Africa	Agonist
Theraphosidae	Pterinochilus	lugardi	female	Africa	Agonist
Theraphosidae	Pterinochilus	murinus	unknown	Africa	NA
Theraphosidae	Pterinochilus	sp. (Kenya)	female	Africa	Agonist
Theraphosidae	Schismatothele	inflata	female	America	NA
Theraphosidae	Schismatothele	sp. (Norte de Santander, Colombia)	female	America	NA
Theraphosidae	Schismatothele	sp. (Tachira, Venezuela)	female	America	NA
Theraphosidae	Selenocosmia	arndsti	female	Asia	NA
Theraphosidae	Selenocosmia	crassipes	female	Asia	Inhibitor
Theraphosidae	Selenocosmia	effera	female	Asia	Inhibitor
Theraphosidae	Selenocosmia	javanensis sumatrana	female	Asia	NA
Theraphosidae	Selenocosmia	peerboomi	female	Asia	Inhibitor
Theraphosidae	Selenocosmia	sp. (Borneo)	female	Asia	Inhibitor
Theraphosidae	Selenocosmia	sp. (Crocker Range, Malaysia)	female	Asia	Inhibitor
Theraphosidae	Selenocosmia	sp. (Malaysia)	female	Asia	NA
Theraphosidae	Selenotholus	foelschei	female	Australia	Inhibitor
Theraphosidae	Selenotypus	plumipes	female	Australia	Inhibitor
Theraphosidae	Sericopelma	silvicola	female	America	Agonist
Theraphosidae	Sericopelma	sp. 1 (Panama)	female	America	NA
Theraphosidae	Sericopelma	sp. 2 (Panama)	female	America	NA
Theraphosidae	Sericopelma	sp. 3 (Panama)	female	America	NA
Theraphosidae	Sericopelma	sp. 4 (Panama)	female	America	NA
Theraphosidae	Sphaerobothria	hoffmanni	female	America	NA
Theraphosidae	Stichoplastoris	sp. (Costa Rica)	female	America	Agonist
Theraphosidae	Stromatopelma	calceatum	female	Africa	NA
Theraphosidae	Tapinauchenius	cupreus	female	America	NA
Theraphosidae	Tapinauchenius	elenae	female	America	NA
Theraphosidae	Tapinauchenius	gigas	female	America	Inhibitor
Theraphosidae	Tapinauchenius	plumipes	female	America	Inhibitor
Theraphosidae	Tapinauchenius	violaceus	female	America	Inhibitor
Theraphosidae	Theraphosa	apophysis	female	America	Inhibitor
Theraphosidae	Theraphosa	blondi	female	America	Inhibitor
Theraphosidae	Thrigmopoeus	truculentus	female	Asia	Agonist
Theraphosidae	Thrixopelma	pruriens	female	America	Inhibitor
Theraphosidae	Xenesthis	immanis	male	America	Agonist
Theraphosidae	Xenesthis	sp. ('blue', Colombia)	female	America	NA
Theraphosidae	Xenesthis	sp. ('white', Colombia)	female	America	Agonist

¹Information in parentheses refers to the collection site or the non-scientific name used in pet trade.

 $^{^2}$ Activity against hNa $_V$ 1.7 in the high-throughput FLIPR assay: venoms are defined as containing an inhibitor or agonist of hNa $_V$ 1.7, or being not active (NA) against this channel.